OPEN ACCESS

University of BRISTOL

Newsome, R. F., Green, M. J., Bell, N. J., Bollard, N. J., Mason, C. S., Whay, B., & Huxley, J. N. (2017). A prospective cohort study of digital cushion and corium thickness, Part 1: associations with body condition, lesion incidence and proximity to calving. *Journal of Dairy Science*, *100*(6), 4745–4758. https://doi.org/10.3168/jds.2016-12012

INTERPRETIVE SUMMARY

Body fat and the digital cushion and corium in lameness

Lesions that result from disruption to claw horn formation on the foot commonly lead to lameness. Cushioning structures within the foot might become depleted with body fat mobilization in early lactation, and have detrimental effects on cow health and productivity. This work found that whilst digital cushion thickness did change with body fat measures over time, other factors, such as calving and lesion incidence also had a great effect on digital cushion thickness. Whilst minimizing body fat loss might help prevent lameness, other physiological events such as calving are also important control points for lameness.

Newsome

A prospective cohort study of digital cushion and corium thickness, Part 1: associations with body condition, lesion incidence and proximity to calving.

R. F. Newsome*[1], M. J. Green*, N. J. Bell†, N. J. Bollard*, C. S. Mason#, H. R. Whay||, J. N. Huxley*

**University of Nottingham, School of Veterinary Medicine and Science, Sutton Bonington Campus, Sutton Bonington, Leicestershire, LE12 5RD, UK*

†*Royal Veterinary College, Hawkshead Lane, North Mymms, Hertfordshire, AL9 7TA, United Kingdom*

#*Scotland's Rural College (SRUC), Kings Buildings, West Mains Road, Edinburgh, EH9 3JG, UK*

||*School of Veterinary Sciences, University of Bristol, Langford House, Langford, BS40 5DU, United Kingdom*

[1]Corresponding author: reuben.newsome@gmail.com

ABSTRACT

Claw horn disruption lesions (CHDL) are a major cause of lameness in dairy cattle and are likely a result of excessive forces being applied to the germinal epithelium that produces claw horn. The digital cushion is a connective tissue structure, containing depots of adipose tissue, that sits beneath the distal phalanx and has been shown to be thicker in fatter cows. Body condition score (BCS) loss is a risk factor for CHDL, and one possible explanation is that fat is mobilised from the digital cushion during negative energy balance, causing the digital cushion to thin and lose force dissipating capacity, leading to disruption of claw horn growth.

This prospective cohort study investigated the association between measures of body fat and sole soft tissues (SST) thickness (a combined measure of the corium and digital cushion beneath the distal phalanx) in a longitudinal manner. SST of 179 cows in two high yielding dairy herds were measured at five assessment points between 8 weeks prior to and 35 weeks post calving. BCS, back fat thickness (BFT) and lesion incidence were recorded. Data were analysed in a 4-level mixed effects regression model, with the outcome being SST thickness beneath the flexor tuberosity of the distal phalanx.

Data from 827 assessment points were available for analysis. The overall mean of SST was 4.99 mm (SD: 0.95). SST was thickest 8 weeks prior to calving (5.22 mm, SD: 0.91) and thinnest one week post-calving (4.68 mm, SD: 0.87), suggesting that there was an effect of calving on SST. BFT was positively correlated with SST in the model with a small effect size (a 10 mm decrease in BFT corresponded with a 0.13 mm decrease in SST), yet the nadir of BFT was 11.0 mm at 9-17 weeks post calving (when SST was ~4.95 mm), rather than occurring with the nadir of SST immediately after calving. SST also varied with other variables, e.g. cows that developed a sole ulcer or severe sole haemorrhage during the study had thinner SST (-0.24 mm), except when a sole ulcer was present, when it was thicker (+0.53 mm).

Cows that developed lesions had a thinner digital cushion prior to the lesion occurrence, which became thickened with sole ulcer presence, perhaps representing inflammation. Further, whilst BFT was correlated with SST over time, SST may also have been influenced by other factors such as integrity of the suspensory apparatus, which could have a major effect on CHDL. Measures of body fat likely contributed to having thin SST, but other factors including calving, herd and lesion presence also had an effect.

Keywords: dairy cow, lameness, body condition, digital cushion

INTRODUCTION

Claw horn disruption lesions (**CHDL**: sole hemorrhage, sole ulcer and white line disease) cause a large proportion of lameness in dairy cattle and have a high rate of recurrence (Hirst et al., 2002; Reader et al., 2011; Green et al., 2014). These diseases are prevalent in developed dairy systems worldwide (Barker et al., 2007; Dippel et al., 2009; Foditsch et al., 2016), impact significantly on cow welfare and farm profitability (Booth et al., 2004; Sogstad et al., 2006; Cha et al., 2010) and have a plethora of associated risk factors (Cramer et al., 2009; Chapinal et al., 2013; Solano et al., 2015). Sole ulcers and sole haemorrhage appear to be different presentations of a similar disease process, which is likely through insult to the germinal epithelium of the sole and poor quality horn production, as a result of inappropriate transfer of forces through the foot (Bicalho and Oikonomou, 2013; Nuss, 2014); white line disease may also precipitate from the same disease process where contusions occur in the soft tissues around the periphery of the base of the foot (Le Fevre et al., 2001; Newsome et al., 2016a).

Epidemiological studies have demonstrated that body condition loss preceded lameness events, whether lameness was defined by visual detection of impaired mobility (Lim et al., 2015; Randall et al., 2015) or by CHDL treatment incidence (Green et al., 2014). The distal phalanx is suspended from the hoof wall by strong ligamentous attachments, referred to as the suspensory apparatus of the distal phalanx, and is supported by the digital cushion, which is a modified layer of the subcutis that is situated beneath the caudal aspect of the distal phalanx. The cushion and associated structures are considered to be important in absorbing impact and dissipating forces during foot strike and limb loading, protecting the germinal epithelium that produces the sole horn (Lischer et al., 2002). Thickness of the digital cushion has been assessed in several studies that used ultrasonography to measure the distance from the inner aspect of the claw horn to the distal surface of the distal phalanx, beneath the flexor tuberosity. The measurement incorporates two tissue layers: the subcutis (i.e. the digital cushion) and the dermis ("corium"). Previous works have termed combined measurements of the two tissue layers "digital cushion thickness", where the measurement was taken beneath the axial aspect of the flexor tuberosity (Bicalho et al., 2009; Machado et al., 2011), or "sole soft tissue thickness", where the measurement was taken in the midline of the sole (Toholj et al., 2013).

Bicalho et al. (2009) reported that body condition score was positively associated with digital cushion thickness. This association could be biologically plausible because the digital cushion contains adipose tissue (Räber et al., 2004; Räber et al., 2006), therefore lipid could be deposited to and mobilized from the digital cushion during periods of positive and negative energy balance. Further, having a thin digital cushion and corium thickness appears to predispose subsequent lameness from CHDL (Machado et al., 2011; Toholj et al., 2013). A possible mechanism for the temporal association between body condition loss and lameness is that fat is mobilized from the digital cushion during negative energy balance, which leads to depletion of the digital cushion, poorer force dissipation of forces during foot strike, greater peak forces on the germinal epithelium, leading to haemorrhage and interrupted epidermal differentiation and cornification, the formation of poor quality sole horn and subsequent lameness. However, previous works assessing the digital cushion and corium have assessed their combined thickness at a single time point (Bicalho et al., 2009; Machado et al., 2011; Toholj et al., 2013), and whether the digital cushion becomes thinner as body fat is mobilized is yet to be demonstrated. This is a key step in demonstrating whether digital cushion depletion with body condition loss is a mechanism by which cows develop CHDL.

The current article presents a prospective cohort study of the sole soft tissues (a combined measure of thickness of the digital cushion and the corium) lameness and lesions, and analyses of associations between sole soft tissue thickness and measures of body fat. The aim of this analysis was to determine how the digital cushion changes throughout lactation and with changes in measures of body fat.

METHODS

Study design

A prospective cohort study assessed the combined thickness of the digital cushion and corium (termed "sole soft tissue thickness") on the hind claws at five time points (termed "assessment points") between approximately 8 weeks prior to and 35 weeks post calving. The null hypothesis stated that sole soft tissue thickness did not vary with measures of body fat. Animals were studied during first, second, third or fourth lactation, from before calving. On the hind feet, the sole soft tissues were measured ultrasonographically and foot lesions were recorded at each assessment point, and cows were locomotion scored fortnightly from calving. Local ethical approval was granted by the University of Nottingham School of Veterinary Medicine and Science Ethical Review Committee.

Timing of assessment points

Animals were enrolled at the first assessment point, which was at approximately 8 weeks prior to their predicted calving date, termed **AP-8**. The second assessment point occurred between 4 and 10 days post-calving and was termed **AP+1** (approximately 1 week post calving). The third assessment point was at 6, 8 or 10 weeks after AP+1 and this period was assigned sequentially within each lactation group, such that cows from each lactation group were studied across the range of likely timings of peak yield. This third assessment point occurred on average 9 weeks post-calving and was termed **AP+9** and the variation in this timing was accounted for by testing a polynomial function of DIM in the statistical analysis.

Assessment points 4 and 5 were 8 and 20 weeks after AP+9, (**AP+17** and **AP+29**, respectively).

Study farms

Two high producing herds were selected and were visited weekly from 13th November 2013 until 19th May 2014. The farms were selected for convenience to ensure ease of access to cows, good handling facilities and willingness of farm managers to accommodate the study. High producing herds were selected since cows in such systems were more likely to undergo body condition change during lactation. Cow data and management systems information are outlined in Table 1. Both farms fed a partial mixed ration. Mixed ration was provided *ad lib* at the feed face, which was based predominantly on maize and whole crop wheat silages on Farm 1 and a combination of alfalfa, and whole crop wheat silages on Farm 2. The aim of mixed ration formulation was to provide for maintenance energy requirements and 30 litres of milk production, and was supplemented with concentrate feed at a rate of 0.45 kg per litre for parity >1 or parity 1 animals producing >26 or >22 litres per day, respectively. The exact formulation of the rations varied throughout the course of the study, but the overall aims of the diet did not vary. An example mixed ration analysis for each farm is shown in Table 1 (Biotal Forage Analysis, Worcester, UK).

All animals on both farms were trimmed by a professional foot trimmer every 4-6 months; the claws on all feet were trimmed if considered to be over-grown. Additionally, lame cows were treated when identified as lame by stockpersons and this method of lameness management continued as normal throughout the study period.

Sample size and subject enrolment

Sample size was estimated based on the data reported by Bicalho et al. (2009). The calculation was based on a 2-sample t-test with alpha = 0.05 and beta = 0.8, and estimated that 108 cows were required in each of two groups to detect a difference in sole soft tissue thickness of 1 mm, which was the difference reported between cows with BCS 2 and 3 in that study. Due to the longitudinal study design in the current study and statistical analyses in multi-level frameworks (see below), this estimate was likely to be conservative and the target was to have at least 150 cows completing all 5 assessment points. Animals were enrolled at approximately 8 weeks prior to calving for their 1st, 2nd, 3rd or 4th lactation, if there was no intention to cull before the end of the subsequent lactation and until the necessary sample size was reached.

Collection of assessment point data

At each assessment point throughout the study, cows were individually restrained in a foot trimming crush (Farm 1: Electric Hoofcare Crush, GDS-Hoofcare, Netherlands; Farm 2: SA35 Cattle Crush, Wopa, UK) and data were collected as follows. Body condition score (**BCS**) on a 1-5 scale with quarter point intervals (Wildman et al., 1982; Edmonson et al., 1989). Additionally, back fat thickness was measured using B-mode ultrasonography (MyLab30 scanner, Esaote Europe B V, Cambridge, UK) with a 5 cm linear transducer set at 7.5 megahertz (resolution: 0.1 mm). Coupling gel was used at all scanning interfaces. The transducer was placed 5 to 10 cm cranial to the tuber ischium, perpendicular to the skin on a line to the tuber coxa, in order to visualize the fascia profunda, as described by Schröder and Staufenbiel (2006). Two images of back fat thickness were obtained from both the left and the right hand side of the cow. Ultrasonograms were saved for measurement of back fat thickness after the study period was complete, when file order was randomised and a blinded observer measured the distance from the external surface of the skin to the fascia profunda, using electronic calipers using the open-source platform Fiji (Schindelin et al., 2012) for the image analysis software ImageJ (Schneider et al., 2012).

Foot lesions were assessed as follows. The hind feet were raised in turn and inspected for overgrowth. If the claw was deemed to be overgrown, a functional foot trim was performed according to a modification of the Dutch Method; a set claw length was not used (Archer et al., 2015) but emphasis was placed on maintaining claw angles (Manske et al., 2002). When a claw was deemed to be in shape, a very thin (<0.5 mm) shaving was removed from the plantar surface of the whole foot (Leach et al., 1998) in order to clearly visualize any lesions present. A photograph was taken of the sole with a 12 megapixel digital camera (Cyber-shot DCS-W510, Sony Europe Ltd, Surrey, UK) held square to the claw, 30 cm distant. Photographs were stored for lesion analysis after the on-farm data collection was complete. Briefly, for the current analysis lesions were categorised as sole ulcer, severe sole haemorrhage, severe white line lesion or a digital dermatitis lesion; Newsome et al. (2016b) describes the full lesion analysis, which was based on lesion descriptors previously utilized in the literature (Dopfer et al., 1997; Leach et al., 1998; Sogstad et al., 2007).

After the base of claws had been photographed, the soft tissues between the distal phalanx and the internal aspect of the sole horn were imaged using ultrasonography, as described by Kofler et al. (1999). The transducer was placed in a standoff and placed on the midline of the claw, such that ultrasonograms of the sole soft tissues could be measured at three sites: (1) the most distal point of the distal phalanx at the toe, (2) the most proximal point of the arch of the distal phalanx and (3) the most distal point beneath the flexor tuberosity (Figure 1). Two replicate images were taken at each site and stored for measurement later. After the study period, image order was randomised as for back fat thickness, and measurements were taken; measurements at these sites included the corium (dermis) at all sites and the digital cushion (subcutis) at sites 2 and 3. The term "sole soft tissue thickness" at sites 1, 2 and 3 is used in this study to describe the thickness of the soft tissues between the sole horn and distal phalanx.

The complete dataset of back fat and sole soft tissue ultrasonographic measurements consisted of 23,598 measurements. Raw data were checked by inspecting and re-measuring outlying data points. Next, 2.5% of ultrasonograms were randomly selected and re-measured; the R-squared value between checked and original was 0.992. "Within-assessment point" repeatability was assessed by comparing replicate measures. R-squared was 0.988 and repeatability was deemed to be very good.

Other data collection

In addition to data collected at each assessment point, withers height was recorded at AP-8. Animal data and production data were collected from farm management software (UNIFORM-Agri, Somerset, UK). Weigh cells were present in the milking robots on Farm 1. On Farm 2, weigh cells (HD1010 Load Bars, Tru-Test Ltd, Auckland, New Zealand) were installed beneath the foot trimming crush and body weight was recorded at each assessment point. Weigh cells were checked throughout the study using known weights and readings remained consistent.

Management of dropouts and missing data

An assessment point was terminated early if a cow became unduly stressed during an assessment, or missed completely if temperament posed a risk to handlers or herself, or for health reasons such as mastitis. If a clear ultrasonographic image could not be obtained, an image of the sole soft tissues was not taken. If a block was present on a claw, the non-blocked claw was still imaged, but no ultrasonographic measurement could be taken from the blocked claw (this occurred at <10 claw assessments). Reasons for missing data and exclusions were recorded and other data collected on that cow at the same or other assessment points were included in analyses where sufficient data were available. If a cow missed ≥3 consecutive assessment points, the cow was excluded from the study.

Summary of terms used in analysis

- Assessment point ("AP" +/- the number of weeks relative to calving) – at which a cow was assessed for back fat thickness, BCS (assessed visually), sole soft tissue thickness and foot lesions.
- Back fat thickness (BFT) – an ultrasonographic measure of back fat over the gluteus medius muscle.
- Claw horn disruption lesion (CHDL) – sole ulcer, severe sole haemorrhage, severe white line haemorrhage or severe white line separation.

- Sole soft tissue thickness, at sites 1-3 – ultrasonographic measures of the soft tissues between the inner margin of the sole and the border of the distal phalanx (Figure 1), taken in the midline of the sole, at:
    - Site 1: Corium thickness beneath the apex of the distal phalanx (the digital cushion is absent at this location).
    - Site 2: Digital cushion and corium thickness beneath the highest point of the arch of the distal phalanx.
    - Site 3: Digital cushion and corium thickness beneath the vertex of the flexor tuberosity of the distal phalanx.

Statistical analysis

Data were initially inspected for trends using charts constructed in Microsoft Excel (2010) and descriptive statistics were calculated in Minitab 17 in order to evaluate patterns in the data, which included Pearson correlation coefficients and chi-square tests.

Mixed-effects linear regression models were constructed to explore relationships between explanatory variables and the outcomes sole soft tissue thickness; two separate models were constructed with the outcome either at site 2 or site 3, since at these two sites the digital cushion was incorporated in the measurement. Models were constructed in MLwiN 2.26 (Rasbash et al., 2012) using iterative generalized least squares algorithms with a forward stepwise procedure and took the format:

$$Y_{ijkl} = \alpha + \beta 1X_{l} + \beta 2X_{kl} + \beta 3X_{jkl} + \beta 4X_{ijkl} + f_{l} + v_{kl} + u_{jkl} + e_{ijkl}$$

$f_l \sim N(0, \sigma^2_f)$

$v_{kl} \sim N(0, \sigma^2_v)$

$u_{jkl} \sim N(0, \sigma^2_u)$

$e_{ijkl} \sim N(0, \sigma^2_e)$

where Y_{ijkl} was the outcome of the four level linear regression model; subscripts i, j, k and l denote the ith repeated measure within the jth assessment of the kth claw of the lth cow respectively, α was the intercept, β1, β2, β3 and β4 represent vectors of coefficients for the fixed effects, X_l, X_{lk}, X_{jkl} and X_{ijkl} represent fixed effect variables at cow, claw, claw-assessment point and repeated measure levels respectively and f_l, v_{kl}, u_{jkl} and e_{ijkl} denote the residual error terms at each level (assumed to be normally distributed with mean 0 and variance σ^2). The cow, claw and claw-assessment point level random effects allowed for any explanatory variable to explain variance only at the level at which it varied, therefore accounting for correlations within the data. Cow level explanatory variables tested included lactation number, farm, withers' height and lesion incidence throughout the study period. Claw level variables identified lateral or medial claw and claw-level lesion incidence throughout the study period. Variables were tested denoting whether cows or claws had displayed a lesion at the start of the study (at AP-8) or at previous assessment points during the study, but no data on lesion incidence prior to the start of the study were available. Claw-assessment point level variables were "Time" (day of total study period, with 13th November 2013 = 1), assessment point number, days in milk (DIM, where day of calving = 0 and 8 weeks prior to calving = -56), back fat thickness, BCS, body weight, lesion presence and corium thickness at site 1. No explanatory variables varied at the repeated measure level (within assessment point), but this level was retained to assess the bottom level variance.

Polynomials of all linear variables and biologically plausible interactions were tested. Dummy variables were used to partition subsets of data that poorly fitted the model where necessary.

All variables were offered to the model and the Wald test was applied to determine whether fixed effects remained in a model, i.e. a variable was significant when the coefficient was $\geq 1.96 \times$ SE ($P \leq 0.05$). Models were checked by inspecting residuals at each level. Data points with high influence were removed from the model and the model was refitted to evaluate changes in model coefficients. The likelihood ratio test was used to compare subsets of models, assessing whether the additional complexity of using additional terms and higher model levels improved model fit (Dohoo et al., 2009).

RESULTS

Overview of the dataset

A total of 827 animal assessments were performed, with data from 179, 176, 163, 157 and 152 cows at each of the five assessment points, respectively. The median number of days from AP-8 to calving was 56 (IQR: 35 to 64) and from calving to AP+1, AP+9, AP+17 and AP+29 was 7 (IQR: 5 to 10), 62 (52 to 74), 118 (107 to 130) and 202 (192 to 215). One hundred and five cows were enrolled on Farm 1 and 74 on Farm 2. By lactation number (1, 2, 3 and 4), 70, 44, 39 and 26 cows were enrolled and 66, 38, 27 and 21 completed the study.

Twenty-seven animals left the study: three were found to be not in calf, one developed obturator paralysis, one developed severe interdigital necrobacillosis, ten became sick and were not assessed for welfare reasons (four had severe mastitis and six had undiagnosed illness), eight were culled (four for not getting back in calf, three for poor production, one for recumbency) and four died (one was diagnosed as an abomasal ulcer and three were not investigated *post mortem*).

Table 2 displays the means and standard deviations of sole soft tissue thickness and back fat thickness, at each assessment point. The nadir of sole soft tissue thickness both for sites 2 and 3 occurred at AP+1, and at AP+9 for sole soft tissue thickness at site 1 (i.e. thickness of the corium at the toe), and the nadir of back fat thickness occurred at AP+9 and AP+17. Pearson correlation coefficients between corium thickness at site 1 and each of sole soft tissue thickness at sites 2 and 3 were 0.29 and 0.15 respectively, and between sole soft tissue thickness at sites 2 and 3 was 0.66. Median BCS was 3.5 (range: 1.5 to 4.5). Back fat thickness and sole soft tissue thickness at sites 1, 2 and 3 are plotted against BCS in Figure 2; back fat thickness and BCS were positively correlated. A 1 unit change in body condition score corresponded with a 10 mm change in back fat thickness between body condition scores of 2.5 and 4.5, whilst the magnitude of the effect was smaller below BCS 2.5. Average body weight of all cows across all assessment points was 647 kg (SD: 72.2).

Mixed-effects linear regression model of sole soft tissue thickness

The dataset consisted of 6,454 measures of sole soft tissue thickness from 3,275 assessments of 716 hind claws of 179 cows. Presented is the final model that had the outcome sole soft tissue thickness at site 3 (Table 3). An alternative model that had the outcome sole soft tissue thickness at site 2 was very similar, and where models differed is described later. The presented model (with the outcome sole soft tissue thickness at site 3, Table 3) had four levels and was selected because model fit was good, because this is the region of the sole ulcer (beneath the flexor tuberosity), and because large variations in sole soft tissue thickness were found with lesion presence. It was therefore considered to present the most information regarding the biology of sole soft tissue thickness, back fat thickness and changes that were evident with CHDL.

The presented model estimated that sole soft tissue thickness on the lateral claw was 0.89 mm greater (CI: 0.84-0.95) than on the medial claw. Cows on Farm 1 had a sole soft tissue thickness 0.27 mm greater (CI: 0.14 to 0.40) than those on Farm 2. Sole soft tissue thickness at AP+1 was 0.33 mm thinner (CI: 0.28 to 0.39) than at other assessment points; this difference was not explained by other variables tested. Withers height and polynomial terms of time (which had a small effect size) were significant and retained in the model.

Sole soft tissue thickness was positively correlated with back fat thickness and several interactions between back fat thickness and other variables were significant. A 10 mm difference in back fat thickness corresponded with a 0.13 mm difference in sole soft tissue thickness, for measures of sole soft tissue thickness at AP-8, +9, +17 and +29, based on the mean corium thickness at site 1 and when no sole ulcer or M2 digital dermatitis lesion was present. Cows that experienced a sole ulcer or a severe sole haemorrhage on any claw at any assessment point had sole soft tissue thickness 0.24 mm thinner (CI: 0.11 to 0.37) than other cows, except when a sole ulcer was present on a claw at an assessment point, when the sole soft tissues were thickened by 0.53 mm (CI: 0.35 to 0.71). Additionally, an interaction showed that the sole soft tissues were particularly thickened when a sole ulcer was present and the cow was thin. To illustrate this, sole soft tissue thickness is plotted against back fat thickness as predictions from the model based on cow-level lesion incidence and claw-assessment point sole ulcer incidence in Figure 3A. Further, when back fat thickness was ≤6 mm (i.e. very thin, corresponding with virtually no subcutaneous fat at this site), sole soft tissue thickness was 0.22 mm thicker (CI: 0.13 to 0.32) than when back fat thickness was >6 mm. (This cut off of 6 mm was selected following visualization of the raw data; using cut offs of 6.5 mm or 7 mm had similar results, but with a smaller effect size. A cut off of 5.5 mm had too few cases and was not significant.) This effect is visible at the 10^{th} percentile of back fat thickness in Figure 3A, where sole soft tissue was thicker than predicted by the rest of the regression line, in cows not displaying a lesion. Sole soft tissues were particularly thickened when a sole ulcer was present later in lactation (when the majority of sole ulcers occurred; 7, 4, 5, 14 and 17 sole ulcers were present at each assessment point, respectively), as demonstrated by a plot of an alternative model in Figure 3B.

An interaction was also present between M2 digital dermatitis lesion presence and back fat thickness (Table 2), and was similar to that between back fat thickness and sole ulcer presence (not plotted). Other interactions demonstrated that back fat thickness and sole soft tissue thickness were *not* correlated at AP+1 (when sole soft tissues were thinnest; plotted in Figure 3C) and that the magnitude of the correlation decreased as sole soft tissue thickness at site 1 became thicker. In the presented model, 61 % of the null model variance remained unexplained. Of this unexplained variance, 48 % was at the claw-assessment point level. Model fit was good.

In the presented model (Table 3), whilst back fat thickness was positively correlated with sole soft tissue thickness at site 3, BCS (observed visually) was *not* correlated with sole soft tissue thickness at site 3. This is despite a strong positive correlation between back fat thickness and BCS (Figure 2). In an alternative model of sole soft tissue thickness at site 3 (not shown), a polynomial term of DIM was significant, but the DIM term correlated with back fat thickness and therefore was excluded from the presented model. In the final model of sole soft tissue thickness at site 2 (not shown), an interaction “back fat thickness ≤6 mm × sole ulcer on a claw at an AP” was not significant, and there was a significant effect of lactation that explained a large degree of the cow-level variance (multiparous animals had a thicker digital cushion at site 2, compared with primiparous animals, data not displayed). This alternative model explained 41% of the null variance of sole soft tissue thickness at site 2, with lactation number explaining much of the cow-level variance. In the presented model (Table 3), no effect of lactation number or primiparous vs multiparous was significant (beyond a significant effect of withers height, which fitted the model well), but otherwise model parameters were similar between the final models for sole soft tissue thickness at site 2 and at site 3.

DISCUSSION

This longitudinal study measured the thickness of the sole soft tissue beneath the distal phalanx – a combined measure of digital cushion and corium thickness – at five time points during the production cycle. Sole soft tissue thickness changed with ultrasonographic measures of back fat thickness throughout lactation, yet the effect size of back fat thickness on sole soft tissue thickness was small in comparison with previous work (Bicalho et al., 2009). Other variables that had an effect on sole soft tissue thickness included lesion occurrence, for example the sole soft tissues was thicker when a sole ulcer was present on a claw, but thinner at other assessment points, and cows that developed either a sole haemorrhage or sole ulcer at any point during the study had thinner sole soft tissues at all assessment points. The sole soft tissues were thinner when an M2 digital dermatitis lesion was present. Thickness of the corium (measured at the apex of the distal phalanx, site 1) had a positive effect on sole soft tissue thickness, likely because the outcome variable includes both the digital cushion and the corium. The sole soft tissues were thicker in taller cows, in cows on Farm 1 and on the lateral claw. Additionally, the sole soft tissue were thinnest immediately after calving (at AP+1, 4-10 days post calving), which was considerably before the nadir of back fat thickness. Addressing the null hypotheses, sole soft tissue thickness changed with back fat thickness, with a small effect size, and many other factors also contributed to thickness of the sole soft tissues.

Sole soft tissue thickness correlated positively with back fat thickness over time, although the observed effect sizes were not of the magnitude reported in previous studies. In work where individual cows were assessed once, Bicalho et al. (2009) reported that a 1 unit difference in BCS corresponded with a 1 mm difference in sole soft tissue thickness. In the current work, a 1 unit difference in BCS (approximately a 10 mm difference in back fat thickness) corresponded with approximately a 0.13 mm difference in sole soft tissue thickness. The absolute thickness also differed: in the current work, the mean sole soft tissue thickness was approximately 50 % thinner than that reported by Bicalho et al. (2009), but was very similar to measurements reported in other work (Kofler et al., 1999; Toholj et al., 2013; Cecen et al., 2015). This could suggest that the scanning site used in the current study was different to that used by Bicalho et al. (2009), who describe a scanning site more axially, whilst in this and in other works (Kofler et al., 1999; Toholj et al., 2013; Cecen et al., 2015) the scanning site was in the midline. Scanning more axially could have targeted a larger depot of fat, explaining differences in correlations with measures of body fat between the studies. Whilst scanning in the midline in the current work found a smaller correlation between back fat thickness and sole soft tissue thickness, this work highlights additional factors that could be important in CHDL development.

A principal finding of the study was that the nadir of sole soft tissue thickness occurred one week post-calving. This could be an effect of peri-parturient hormones, such as relaxin (Tarlton et al., 2002) or oestrogens. Relaxin, for example, mediates distension of the reproductive tract for parturition by activating metalloproteinases that degrade collagen and is known to have effects on other structures throughout the body (Samuel et al., 1998); if it acts upon the suspensory apparatus it could cause the distal phalanx to sit lower in the hoof around calving. In previous work assessing the thickness of the sole soft tissues in a cross sectional study, the nadir of sole soft tissue thickness was observed at approximately 120 DIM and corresponded with the nadir of BCS (Bicalho et al., 2009). This discrepancy between the two works could have arisen because Bicalho et al. (2009) measured the sole soft tissues within 30 DIM, by which time the suspensory apparatus may have regained integrity if laxity was only temporary. Alternatively, farm management systems were very different between the current study and (Bicalho et al., 2009); walking distances were not recorded but cow activity could explain some of the differences seen. Furthermore, in the current study back fat thickness was not positively correlated with sole soft tissue thickness immediately after calving (at AP+1, Figure 3C), suggesting that thickness of the sole soft tissues is not related to measures of body fat at this time. These findings highlight that our measurement of sole soft tissue thickness reflected the position of the distal phalanx within the hoof, which was a function of both back fat thickness and integrity of the suspensory apparatus. This could highlight the importance of the suspensory apparatus on the position of the distal phalanx within the hoof capsule and its importance in lesion pathogenesis.

Sole soft tissue thickness was thicker when a sole ulcer was present. We propose that this may have been due to inflammation in the underlying tissues. In previous work that scanned the sole soft tissues within 4 to 10 days after calving, the soles of feet in cows without lesions were hotter if the sole soft tissues were thinner. The authors hypothesized that reduced sole soft tissue thickness was associated with trauma in the region and early signs of inflammation, before CHDL became visible (Oikonomou et al., 2014b); this thinness could have been predisposed by laxity of the suspensory apparatus. Such results could suggest that vascular or inflammatory changes occur within the soft tissues of the sole of the foot in lesion development. Additionally, previous work has demonstrated increased new bone growth on the flexor tuberosity of the distal phalanx in cows that have suffered more lameness and CHDL throughout life, and one possible mechanism for this new bone growth is inflammation in the surrounding soft tissues with CHDL (Newsome et al., 2016a). Previous work has also shown that combining the administration of NSAIDs with applying a block to the non-affected claw improved recovery rates for lameness in acute cases of disease (Thomas et al., 2015). The fact that the sole soft tissues appear to have been inflamed when a sole ulcer was present, and the potential detrimental effects this has on the surrounding anatomical structures such as the flexor tuberosity, highlights the importance firstly of prevention, and secondly of early detection and effective treatment of lame cows, which current evidence suggests should include the administration of NSAIDs and the application of a block to the non-lame claw.

Cows that developed a sole ulcer or a severe sole haemorrhage during the study had thinner sole soft tissues on all claws than other cows (except when a claw had a sole ulcer, when the sole soft tissues of that claw were thickened). This cow-level effect was not explained by the stature or milk production variables tested and could be an effect of genotype or phenotype: cows with thin digital cushions were more likely to develop lesions, possibly as a result of decreased force dissipating capacity. Additionally, it could reflect rearing differences, as the digital cushions of calves have been found to develop larger with more mechanical challenge before 6 months of age (Gard et al., 2015). Thirdly, it could reflect prior unrecorded CHDL, with the digital cushion thinning after insult (Lischer et al., 2002). Whilst the current study cannot confirm what caused the thinness of the sole soft tissues prior to lesion development, it highlights that maximizing the thickness of the digital cushion could have a beneficial effect on foot health. Two possible mechanisms for this could be to (1) select for thickness of the digital cushion in breeding programs (Oikonomou et al., 2014a), or (2) manipulate rearing systems in order to optimize the structure and function of the digital cushion prior to first calving. Altering rearing systems could prove to be highly beneficial in reducing life time CHDL risk and is an interesting area for future research.

An interesting finding of this work is that the sole soft tissues were thinner when an M2 digital dermatitis lesion was present. It is unclear how the presence of such an infection might cause thinning of the dermis and subcutis, yet the association could be due to either unidentified causal or non-causal reasons. The presence of digital dermatitis could indicate a socially subordinate cohort of animals that spent longer standing, and as a result had thinner digital cushions. Alternatively, a cow's predisposition to digital dermatitis might be a function of a physiologic state that also causes laxity in the suspensory apparatus and a thinner digital cushion. Such inter-relationships between all causes of lameness, standing time, physiologic state and hoof anatomy clearly warrant further study.

This study was based on two high yielding herds that were housed year-round and may not be representative of the dairy cow population at a whole. However, the study cows did lose significant amounts of condition during early lactation as would be expected in high yielding cows, therefore it was likely a suitable population in which to look for changes in thickness of the sole soft tissues with body fat change. It was difficult to fully assess associations between measures of body fat and digital cushion thickness because other variables, such as integrity of the suspensory apparatus, appeared to influence sole soft tissue thickness. Further, whilst ultrasonography can precisely measure the thickness of the sole soft tissues beneath the distal phalanx (Kofler et al., 1999; Bicalho et al., 2009; Cecen et al., 2015), and high specification machines as used in this study can do so with high precision, it might not to be a good indicator of adipose content within the digital cushion. Recent work has found that non-pregnant dairy cows fed a higher energy diet prior to slaughter had greater upregulation of lipogenic genes within the digital cushion (Iqbal et al., 2016), but how negative energy balance or broader physiologic state interact with lipolytic pathways and mobilisation of fat from the digital cushion is still unclear.

Finally, it must be noted that the study herds had very low white line lesion incidences (see Newsome et al. (2016b)). Therefore, whilst no variable describing white line lesion incidence was significant in the current study, the dataset may have lacked sufficient power to identify such differences. It remains possible that differences in sole soft tissue thickness exist between cows or claws that develop white line lesions and this should be investigated in herds with a higher incidence of these lesions.

CONCLUSIONS

This longitudinal study found that sole soft tissue thickness was positively correlated with repeated measures of body fat over time. However, the effect of back fat thickness on sole soft tissue thickness was much smaller than reported in previous work and there were multiple exceptions to this correlation. The sole soft tissues were thinnest immediately after calving and did not correlate with back fat thickness at this assessment point; this could have been an effect of hormonal influences surrounding calving. Cows that developed either a sole ulcer or a severe sole haemorrhage had thinner digital cushions, yet when a sole ulcer was present the soft tissues on that claw were thickened, which could have been a result of increased vascularization, oedema or inflammation in the underlying tissues. Measures of body fat appeared to be one component that could contribute to having a thin digital cushion, but other factors played a part, including an effect of calving and other cow-level effects. Further work should explore the extent to which thinning of the sole soft tissues, and absolute thinness, influences CHDL, and should also identify the proportion of CHDL that are a result of body condition loss, with a view to working out whether managing body condition loss might reduce lameness.

ACKNOWLEDGEMENTS

This work constituted part of a doctoral thesis by the first author, which contains further validation and analysis. A digital version of the thesis will be available from http://eprints.nottingham.ac.uk late in 2017. The work was funded by the Agriculture and Horticulture Development Board (AHDB) Dairy Division, a levy board, not for profit organisation working on behalf of British Dairy Farmers. The authors thank Nikki Bollard and Katie Holmes for technical support throughout the project and farm staff for accommodating the study.

FIGURES AND TABLES

Table 1: Farm systems and animal data for two study farms used in a prospective cohort study of the digital cushion, hoof lesions and lameness.

Variable	Farm 1	Farm 2
Housing		
Milking system	4 × Lely A3 automatic milking systems	4 × Lely A4 automatic milking systems
Management groups (randomly assigned)	4 groups, 1 robot per group	2 groups, 2 robots per group
Number of cubicles	241	240
Total floor area	1,196 m^2 (excl. cubicles)	1,016 m^2 (excl. cubicles)
Floor type	Rubber matting	Concrete slats
Shed roof type	Pitched, open ridge	Pitched, open ridge
Shed ventilation	Combination of natural and fan assisted ventilation	Natural ventilation via side-walls
Pre-calving heifer housing	Cubicle sheds from 6 months old, with rubber mats in cubicles and concrete passageways	At pasture during spring, summer and autumn months from 6 months old, or indoors on deep straw bedding, weather dependent
Cubicle dimensions		
Width	1.16 m	1.12 m
Neck rail height	1.2 m	1.3 m
Length to brisket board	1.75 m	1.85 m
Kerb height	0.2 m	0.16 m
Management		
Foot bathing protocol	3 times per week: 2 × 4% formalin solution, 1 × 5% copper sulfate solution	Fortnightly, alternating between 4% formalin solution and 5% copper sulfate solution
Scraper frequency	Once every hour	Once every hour
Animal data		
No. of cows milking	Average: 175 (Max: 190)	Average: 201 (Max: 210)
Breed	100% Holstein	≥ 75% Holstein genetics. Brown Swiss and Ayreshires had been crossed into the herd.
Age at 1st calving[1]	Mean: 25.8 mo (median: 25.6)	26.8 mo (26.7)
Milk frequency, per day[1]	2.9	3.5
Mean farm 305d yield[2]	11,380 kg	12,350 kg
Calving interval[1]	Mean: 366 d (median: 394)	401 d (411)
Lactation length[1]	305 d (310)	311 d (308)
Feeding information		
Feed type	Partial mixed ration: mixed ration *ad lib* at feed face, supplemented with concentrates to production in parlour.	Partial mixed ration: mixed ration *ad lib* at feed face, supplemented with concentrates to production in parlour.
Feed frequency (ration)	1 per day	1 per day
Push-up frequency	6 per day	11 per day
Analysis of mixed ration[2]		
Dry matter (%)	38	39
ME (MJ/kg of DM)	12.1	12.4
CP (g/kg of DM)	160	181
Sugar (g/kg of DM)	32	15
Starch (g/kg of DM)	270	205
NDF (g/kg of DM)	415	480
Oil (g/kg of DM)	55	60
Feed space length/ cow	0.83 m	0.63 m
Feed space partitioning	184 headlocks	192 headlocks
Water points	2m × 0.6m water troughs (n = 18)	2m × 0.6m water troughs (n = 16)

[1]Animal data that applies to animals studied.
[2]Data measured at end of study, for variables that varied over time.

Table 2: Ultrasonographic measurement data collected at five assessment points during a prospective cohort study of sole soft tissue thickness (measured at three sites) in dairy cows.

AP[1]	Back fat thickness, mm (SD, n[2])	BCS Median	BCS Upper quart		BCS Lower quart	Sole soft tissue thickness, mm (SD, n[3]) Site 1: corium only	Site 2: digital cushion and corium[4]	Site 3: digital cushion and corium[5]
–8	18.9 (5.7, 170)	3.5	3.5	-	4	3.71 (0.67, 674)	7.43 (1.04, 671)	5.22 (0.91, 670)
+1	16.6 (5.9, 175)	3.5	3	-	3.75	3.57 (0.69, 696)	7.24 (0.98, 695)	4.68 (0.87, 696)
+9	11.1 (5.0, 167)	3.25	2.75	-	3.5	3.21 (0.60, 661)	7.36 (1.08, 661)	4.89 (0.90, 660)
+17	10.9 (5.3, 163)	3.25	3	-	3.5	3.35 (0.57, 641)	7.47 (1.03, 639)	5.02 (0.96, 639)
+29	13.3 (5.8, 152)	3.25	3	-	3.75	3.49 (0.60, 603)	7.68 (1.02, 599)	5.20 (0.97, 597)
All data	14.3 (6.4, 827)	3.5	3	-	3.75	3.47 (0.67, 3,275)	7.43 (1.06, 3,265)	4.99 (0.95, 3,262)

[1] Assessment point, weeks relative to calving

[2] Number of cows measured; two repeat measures taken on each side of the cow (left and ride) at each assessment point

[3] Number of claws measured; two repeat measures taken at each site at each assessment point

[4] Beneath the apex of the distal phalanx

[5] Beneath the flexor tuberosity of the distal phalanx

Table 3: A linear regression model of sole soft tissue thickness (**SST**) beneath the flexor tuberosity of the distal phalanx, measured during a prospective cohort study of 179 dairy cows.

Response:	Mean (SD)[1]	No. of units[2]	Sole soft tissue thickness at site 3 (mm) Coefficient	Lower 95% CI	Upper 95% CI
Fixed Part					
Intercept			4.69		
Assessment Point (AP)$_j$					
AP-8, +9, +17 or +29		2,579	Baseline		
AP+1		696	-0.335	-0.389	-0.282
Claw$_k$					
Medial		358	Baseline		
Lateral		358	0.892	0.836	0.948
Farm$_l$					
1		105	Baseline		
2		74	-0.269	-0.403	-0.135
BFT$_j$ (categorical)					
>6 mm		2,991	Baseline		
≤6 mm		284	0.221	0.123	0.319
SST at site 1 (toe)$_j$, mm	3.47 (0.65)		0.101	0.0603	0.141
Withers height$_l$, cm	144 (4.08)		0.0360	0.0197	0.0522
Cow SU/SevSH incidence$_l$					
Never occurred		147	Baseline		
Occurred		32	-0.237	-0.370	-0.105
Sole ulcer$_j$					
Absent		3228	Baseline		
Present		47	0.531	0.349	0.714
DD M2 lesion$_j$					
Absent		3233	Baseline		
Present		42	-0.223	-0.411	-0.0351
BFT$_j$ (continuous), mm[3]	14.3 (6.4)		0.0132	0.00687	0.0195
Interactions with BFT$_j$[4]					
BFT$_j$ × AP+1$_j$			-0.184	-0.268	-0.100
BFT$_j$ × SST at site 1 (toe)$_j$			-0.137	-0.192	-0.0819
BFT$_j$ × sole ulcer present$_j$			-0.761	-1.02	-0.502
BFT$_j$ × DD M2 lesion present$_j$			-0.605	-0.974	-0.235
Random Part			σ2 (SE)	% remaining at each level	
Level:					
l: Cow		179	0.161 (0.021)	28.6%	
k: Claw		716	0.075 (0.009)	13.4%	
j: Claw-Assessment Point		3,275	0.270 (0.008)	47.9%	
i: Repeated measure		6,454	0.057 (0.001)	10.1%	
Total variance:			Remaining: 0.566	Explained: 38.7%	

Cows were assessed at 5 assessment points (**AP**) between 8 weeks prior to and 29 weeks post calving. Explanatory variables included continuous and categorical terms of ultrasonographic measures of back fat thickness (**BFT**), cow-level occurrence of either a sole ulcer or a severe sole haemorrhage during the study period (Cow SU/SevSH incidence), claw-assessment point level sole ulcer occurrence ("sole ulcer"), presence of an M2 DD lesion, sole soft tissue thickness at site 1 (the toe), other variables shown named and interactions between variables are shown. Subscripts i, j, k and l denote the lowest level of the model at which a term varied. Linear terms are centred around the grand mean.
Time (duration throughout study) was included to the fourth polynomial and had a small effect size; coefficients omitted.
Terms are significant when the 95% confidence interval does not include 0 (Wald Test, α = 0.05).
[1]Mean and standard deviation for continuous variables. [2]Number of units in each category, for categorical variables.
[3]Coefficients for continuous back fat thickness measurements relate to a 10 mm difference.
[4]The baseline of each interaction term is the baseline for the coefficient not in the interaction, when back fat thickness = 0.

Figure 1:

Top: Ultrasonogram of back fat. The transducer was placed 5 to 10 cm cranial to the tuber ischium, perpendicular to the skin on a line to the tuber coxa, in order to visualize the fascia profunda. “Back fat thickness” was measured from the external surface of the skin to the fascia profunda in the midline of each image, as described by Schröder and Staufenbiel (2006).

Middle: Midline sagittal section of a bovine digit (left), with the distal phalanx and the digital cushion (DC) outlined. Vertical black lines indicate the three measurement sites of sole soft tissue, extending from the inner margin of the sole horn to the distal border of the distal phalanx in the midline. Site 1 includes only the corium. Sites 2 and 3 measure both digital cushion and corium thickness, and the landmarks for the measurements are the highest point of the arch beneath the distal phalanx and the vertex of the flexor tuberosity, respectively. A red square marks the region in which the sole soft tissues were imaged at sites 2 and 3 using ultrasonography.

Bottom: Ultrasonogram of the sole soft tissues.

Figure 2. Sole soft tissue thickness measured at three sites and back fat thickness plotted against BCS, for all data collected during a prospective cohort study of sole soft tissue thickness and measures of body fat. Measurements were taken at 5 assessment points; all data are at the claw-assessment point level. Mean and standard error are shown. The numbers of sole soft tissue measurements for each BCS score (1.5 to 4.5, with quarter-point intervals between 2 and 4) were 4, 13, 20, 41, 55, 117, 123, 207, 139, 74 and 29 respectively. The back fat thickness measurement includes skin thickness, which is approximately 5 mm thick,

therefore back fat thickness measures of this magnitude represent virtually no subcutaneous fat being present at the site. Standard error bars are shown.

Figure 3. Predictions of sole soft tissue thickness at site 3 from linear regression models of data collected during a prospective cohort study. A and C were based on the reported model (Table 3) and B was based on an alternative model that included “Assessment Point” as a categorical fixed effect and appropriate interactions. Predictions were taken based on no M2 digital dermatitis lesion being present. Error bars show 95% confidence intervals.

A) Sole soft tissue thickness is plotted against deciles of back fat thickness (absolute BFT is shown). Different lines demonstrate different groups of data, as follows: (1) cows that did not develop a sole ulcer or severe sole haemorrhage during the study, (2) cows that did develop a sole ulcer or severe sole haemorrhage during the study and a sole ulcer was not present on the claw at the assessment point, and (3) sole ulcer present on the claw at the assessment point. Predictions were based on sole soft tissue thickness at AP-8, AP+9, AP+17 and AP+29 (i.e. not AP+1 when BFT was not correlated with sole soft tissue thickness). The numbers of sole ulcers that occurred within each decile were 11, 7, 4, 1, 2, 3, 3, 7, 5 and 4. The numbers of severe sole haemorrhages within each decile were 22, 28, 26, 18, 19, 8, 12, 5, 9 and 5.

B) Sole soft tissue thickness plotted by assessment point, against days in milk, with the same data groups as in Figure 3A. The sole soft tissues of claws displaying a sole ulcer were significantly thicker at AP+9, AP+17 and AP+29 than the sole soft tissues of cows that developed a sole ulcer or severe sole haemorrhage during the study but did not display a sole ulcer at that assessment point. The number of sole ulcers present on all claws studied at each assessment point were 7, 4, 5, 14 and 17 respectively.

C) Sole soft tissue thickness is plotted against back fat thickness (mean and +1 and -1 standard deviations are shown). Different lines demonstrate the following data groups: either data taken at AP+1, or at all other assessment points. There was a positive correlation between sole soft tissue thickness at site 3 and back fat thickness at all assessment points, except AP+1. Additionally, sole soft tissue thickness was thinner at AP+1 (immediately after calving) than at other assessment points. This prediction was based on the model assuming no sole soft tissue when no sole ulcers were present.

REFERENCES

Archer, S. C., R. Newsome, H. Dibble, C. J. Sturrock, M. G. G. Chagunda, C. S. Mason & J. N. Huxley 2015. Claw length recommendations for dairy cow foot trimming. Vet. Rec. http://dx.doi.org/10.1136/vr.103197

Barker, Z. E., J. R. Amory, J. L. Wright, R. W. Blowey & L. E. Green 2007. Management factors associated with impaired locomotion in dairy cows in England and Wales. J. Dairy Sci. 90:3270-3277. http://dx.doi.org/10.3168/jds.2006-176

Bicalho, R. C., V. S. Machado & L. S. Caixeta 2009. Lameness in dairy cattle: A debilitating disease or a disease of debilitated cattle? A cross-sectional study of lameness prevalence and thickness of the digital cushion. J. Dairy Sci. 92:3175-84. http://dx.doi.org/10.3168/jds.2008-1827

Bicalho, R. C. & G. Oikonomou 2013. Control and prevention of lameness associated with claw lesions in dairy cows. Livest. Sci. 156:96-105. http://dx.doi.org/10.1016/j.livsci.2013.06.007

Booth, C. J., L. D. Warnick, Y. T. Grohn, D. O. Maizon, C. L. Guard & D. Janssen 2004. Effect of lameness on culling in dairy cows. J. Dairy Sci. 87:4115-4122.

Cecen, G., H. Salci, D. S. Intas, N. Celimli & G. U. Caliskan 2015. Ultrasonographic and macroscopic comparison of the thickness of the capsule, corium, and soft tissues in bovine claws: an in vitro study. J. Vet. Sci. 16:107-12. http://dx.doi.org/10.4142/jvs.2015.16.1.107

Cha, E., J. A. Hertl, D. Bar & Y. T. Grohn 2010. The cost of different types of lameness in dairy cows calculated by dynamic programming. Prev. Vet. Med. 97:1-8. http://dx.doi.org/10.1016/j.prevetmed.2010.07.011

Chapinal, N., A. K. Barrientos, M. A. von Keyserlingk, E. Galo & D. M. Weary 2013. Herd-level risk factors for lameness in freestall farms in the northeastern United States and California. J. Dairy Sci. 96:318-28. http://dx.doi.org/10.3168/jds.2012-5940

Cramer, G., K. D. Lissemore, C. L. Guard, K. E. Leslie & D. F. Kelton 2009. Herd-level risk factors for seven different foot lesions in Ontario Holstein cattle housed in tie stalls or free stalls. J. Dairy Sci. 92:1404-1411. http://dx.doi.org/10.3168/jds.2008-1134

Dippel, S., M. Dolezal, C. Brenninkmeyer, J. Brinkmann, S. March, U. Knierim & C. Winckler 2009. Risk factors for lameness in freestall-housed dairy cows across two breeds, farming systems, and countries. J. Dairy Sci. 92:5476-5486. http://dx.doi.org/10.3168/jds.2009-2288

Dohoo, I. R., W. Martin & H. Stryhn 2009. Model-Building Stragies. In: MCPIKE, M. (ed.) Veterinary Epidemiologic Research. 2 ed. Canada: VER Inc.

Dopfer, D., A. Koopmans, F. A. Meijer, I. Szakall, Y. H. Schukken, W. Klee, R. B. Bosma, J. L. Cornelisse, A. vanAsten & A. terHuurne 1997. Histological and bacteriological evaluation of digital dermatitis in cattle, with special reference to spirochaetes and Campylobacter faecalis. Vet. Rec. 140:620-623.

Edmonson, A. J., I. J. Lean, L. D. Weaver, T. Farver & G. Webster 1989. A Body Condition Scoring Chart for Holstein Dairy-Cows. J. Dairy Sci. 72:68-78.

Foditsch, C., G. Oikonomou, V. S. Machado, M. L. Bicalho, E. K. Ganda, S. F. Lima, R. Rossi, B. L. Ribeiro, A. Kussler & R. C. Bicalho 2016. Lameness Prevalence and

Risk Factors in Large Dairy Farms in Upstate New York. Model Development for the Prediction of Claw Horn Disruption Lesions. PLoS ONE 11:e0146718. http://dx.doi.org/10.1371/journal.pone.0146718

Gard, J. A., D. R. Taylor, D. R. Wilhite, S. P. Rodning, M. L. Schnuelle, R. K. Sanders, R. J. Beyers, M. A. Edmondson, F. J. DeGraves & E. van Santen 2015. Effect of exercise and environmental terrain on development of the digital cushion and bony structures of the bovine foot. Am. J. Vet. Res. 76:246-252. http://dx.doi.org/10.2460/ajvr.76.3.246

Green, L. E., J. N. Huxley, C. Banks & M. J. Green 2014. Temporal associations between low body condition, lameness and milk yield in a UK dairy herd. Prev. Vet. Med. 113:63-71. http://dx.doi.org/10.1016/j.prevetmed.2013.10.009

Hirst, W. M., R. D. Murray, W. R. Ward & N. P. French 2002. A mixed-effects time-to-event analysis of the relationship between first-lactation lameness and subsequent lameness in dairy cows in the UK. Prev. Vet. Med. 54:191-201.

Iqbal, Z. M., H. Akbar, A. Hosseini, E. Bichi Ruspoli Forteguerri, J. S. Osorio & J. J. Loor 2016. Digital Cushion Fatty Acid Composition and Lipid Metabolism Gene Network Expression in Holstein Dairy Cows Fed a High-Energy Diet. PLoS ONE 11:e0159536. http://dx.doi.org/10.1371/journal.pone.0159536

Kofler, J., P. Kubber & W. Henninger 1999. Ultrasonographic imaging and thickness measurement of the sole horn and the underlying soft tissue layer in bovine claws. Vet. J. 157:322-331.

Le Fevre, A. M., D. N. Logue, J. E. Offer, I. McKendrick & G. Gettinby 2001. Correlations of measurements of subclinical claw horn lesions in dairy cattle. Vet. Rec. 148:135-138.

Leach, K. A., D. N. Logue, J. M. Randall & S. A. Kempson 1998. Claw lesions in dairy cattle: methods for assessment of sole and white line lesions. Vet. J. 155:91-102.

Lim, P. Y., J. N. Huxley, J. A. Willshire, M. J. Green, A. R. Othman & J. Kaler 2015. Unravelling the temporal association between lameness and body condition score in dairy cattle using a multistate modelling approach. Prev. Vet. Med. 118:370-7. http://dx.doi.org/10.1016/j.prevetmed.2014.12.015

Lischer, C. J., P. Ossent, M. Räber & H. Geyer 2002. Suspensory structures and supporting tissues of the third phalanx of cows and their relevance to the development of typical sole ulcers (Rusterholz ulcers). Vet. Rec. 151:694-698.

Machado, V. S., L. S. Caixeta & R. C. Bicalho 2011. Use of data collected at cessation of lactation to predict incidence of sole ulcers and white line disease during the subsequent lactation in dairy cows. Am. J. Vet. Res. 72:1338-1343. http://dx.doi.org/10.2460/ajvr.72.10.1338

Manske, T., J. Hultgren & C. Bergsten 2002. The effect of claw trimming on the hoof health of Swedish dairy cattle. Prev. Vet. Med. 54:113-129.

Newsome, R., M. J. Green, N. J. Bell, M. G. G. Chagunda, C. S. Mason, C. J. Sturrock, H. R. Whay & J. N. Huxley 2016a. Linking Bone Development on the caudal aspect of the Distal Phalanx with Lameness during Life. J. Dairy Sci. 99:4512–4525. http://dx.doi.org/10.3168/jds.2015-10202

Newsome, R. F., M. J. Green, N. J. Bell, C. S. Mason, H. R. Whay & J. N. Huxley 2016b. A prospective cohort study of the digital cushion and corium, Part 2: does thinning of the digital cushion and corium lead to lameness and claw horn disruption lesions? J. Dairy Sci. In Review.

Nuss, K. The role of biomechanical factors in the development of sole ulcer in dairy cattle. 1-11 in Cattle Lameness Conference, Worcester. The Dairy Group, Taunton, UK.

Oikonomou, G., G. Banos, V. Machado, L. Caixeta & R. C. Bicalho 2014a. Short communication: Genetic characterization of digital cushion thickness. J. Dairy Sci. 97:532-536.

Oikonomou, G., P. Trojacanec, E. K. Ganda, M. L. S. Bicalho & R. C. Bicalho 2014b. Association of digital cushion thickness with sole temperature measured with the use of infrared thermography. J. Dairy Sci. 97:4208-4215. http://dx.doi.org/10.3168/jds.2013-7534

Räber, M., C. J. Lischer, H. Geyer & P. Ossent 2004. The bovine digital cushion - a descriptive anatomical study. Vet. J. 167:258-64.

Räber, M., M. R. L. Scheeder, P. Ossent, C. J. Lischer & H. Geyer 2006. The content and composition of lipids in the digital cushion of the bovine claw with respect to age and location - a preliminary report. Vet. J. 172:173-7.

Randall, L. V., M. J. Green, M. G. G. Chagunda, C. Mason, S. C. Archer, L. E. Green & J. N. Huxley 2015. Low body condition predisposes cattle to lameness: An 8-year study of one dairy herd. J. Dairy Sci. 98:3766-3777. http://dx.doi.org/10.3168/jds.2014-8863

Rasbash, J., F. Steele, W. J. Browne & H. Goldstein 2012. A User's Guide to MLwiN, v2.26. Centre for Multilevel Modelling, University of Bristol.

Reader, J. D., M. J. Green, J. Kaler, S. A. Mason & L. E. Green 2011. Effect of mobility score on milk yield and activity in dairy cattle. J. Dairy Sci. 94:5045-5052. http://dx.doi.org/10.3168/jds.2011-4415

Samuel, C. S., J. P. Coghlan & J. F. Bateman 1998. Effects of relaxin, pregnancy and parturition on collagen metabolism in the rat pubic symphysis. J. Endocrinol. 159:117-125. http://dx.doi.org/10.1677/joe.0.1590117

Schindelin, J., I. Arganda-Carreras, E. Frise, V. Kaynig, M. Longair, T. Pietzsch, S. Preibisch, C. Rueden, S. Saalfeld, B. Schmid, J.-Y. Tinevez, D. J. White, V. Hartenstein, K. Eliceiri, P. Tomancak & A. Cardona 2012. Fiji: an open-source platform for biological-image analysis. Nat. Meth. 9:676-682. http://dx.doi.org/10.1038/nmeth.2019

Schneider, C. A., W. S. Rasband & K. W. Eliceiri 2012. NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9:671-5.

Schröder, U. J. & R. Staufenbiel 2006. Invited review: Methods to determine body fat reserves in the dairy cow with special regard to ultrasonographic measurement of backfat thickness. J. Dairy Sci. 89:1-14. http://dx.doi.org/10.3168/jds.S0022-0302(06)72064-1

Sogstad, A. M., O. Osteras & T. Fjeldaas 2006. Bovine claw and limb disorders related to reproductive performance and production diseases. J. Dairy Sci. 89:2519-2528.

Sogstad, A. M., O. Osteras, T. Fjeldaas & A. O. Refsdal 2007. Bovine claw and limb disorders at claw trimming related to milk yield. J. Dairy Sci. 90:749-759.

Solano, L., H. W. Barkema, E. A. Pajor, S. Mason, S. J. LeBlanc, J. C. Zaffino Heyerhoff, C. G. R. Nash, D. B. Haley, E. Vasseur, D. Pellerin, J. Rushen, A. M. de Passillé & K. Orsel 2015. Prevalence of lameness and associated risk factors in Canadian Holstein-

Friesian cows housed in freestall barns. J. Dairy Sci. 98:6978-6991. http://dx.doi.org/10.3168/jds.2015-9652

Tarlton, J. F., D. E. Holah, K. M. Evans, S. Jones, G. R. Pearson & A. J. F. Webster 2002. Biomechanical and histopathological changes in the support structures of bovine hooves around the time of first calving. Vet. J. 163:196-204. http://dx.doi.org/10.1053/tvjl.2001.0651

Thomas, H. J., G. G. Miguel-Pacheco, N. J. Bollard, S. C. Archer, N. J. Bell, C. Mason, O. J. R. Maxwell, J. G. Remnant, P. Sleeman, H. R. Whay & J. N. Huxley 2015. Evaluation of treatments for claw horn lesions in dairy cows in a randomized controlled trial. J. Dairy Sci. 98:4477-4486. http://dx.doi.org/10.3168/jds.2014-8982

Toholj, B., M. Cincović, M. Stevančević, J. Spasojevic, V. Ivetić & A. Potkonjak 2013. Evaluation of ultrasonography for measuring solar soft tissue thickness as a predictor of sole ulcer formation in Holstein-Friesian dairy cows. Vet. J. http://dx.doi.org/10.1016/j.tvjl.2013.11.005

Wildman, E. E., G. M. Jones, P. E. Wagner, R. L. Boman, H. F. Troutt & T. N. Lesch 1982. A Dairy-Cow Body Condition Scoring System and Its Relationship to Selected Production Characteristics. J. Dairy Sci. 65:495-501.

Figure 1:

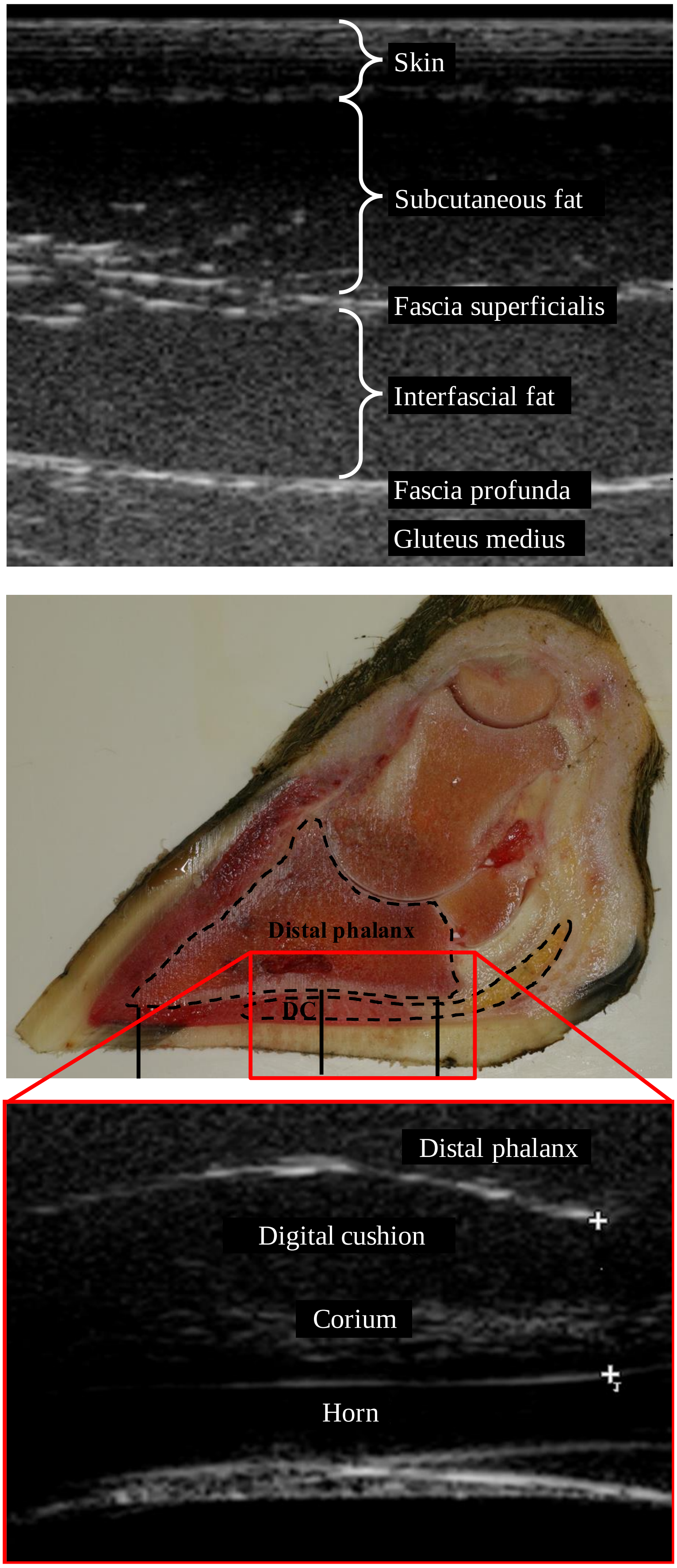
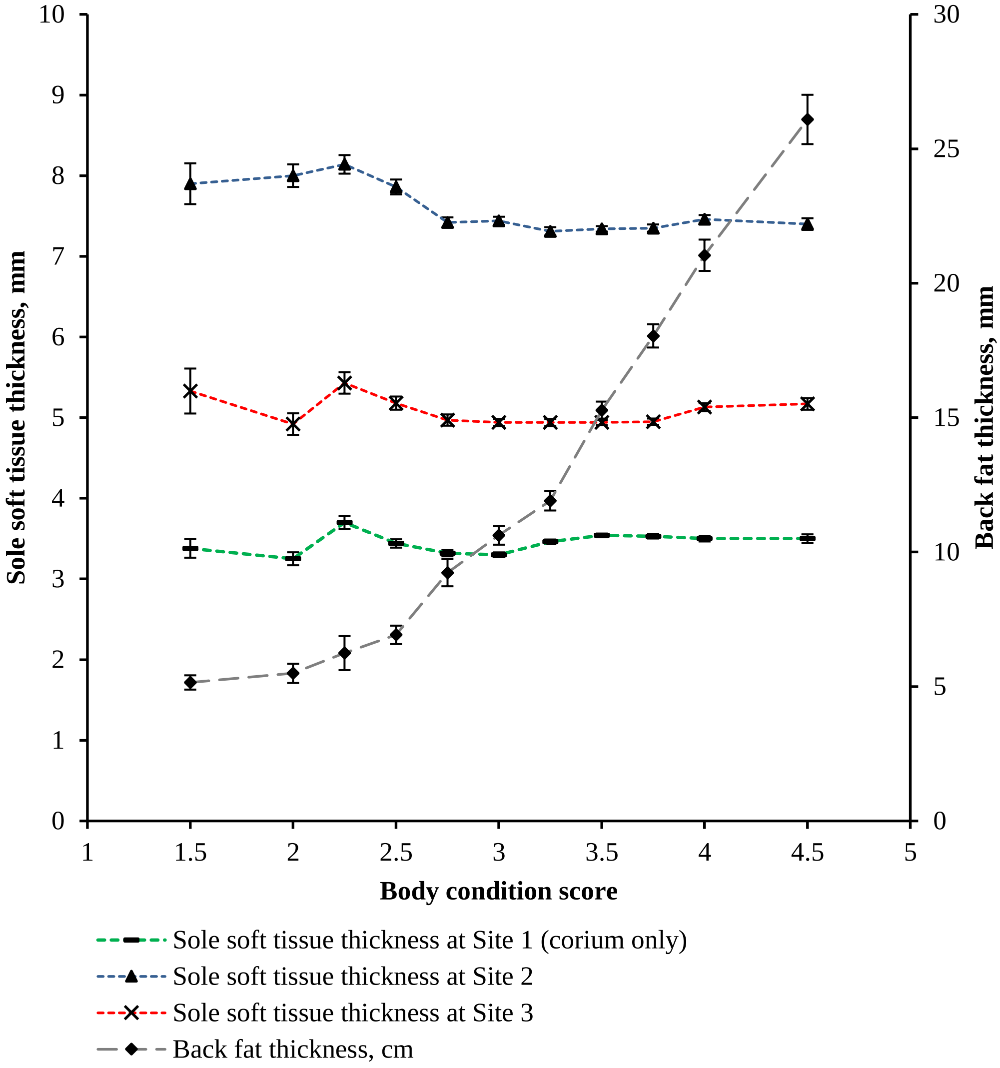
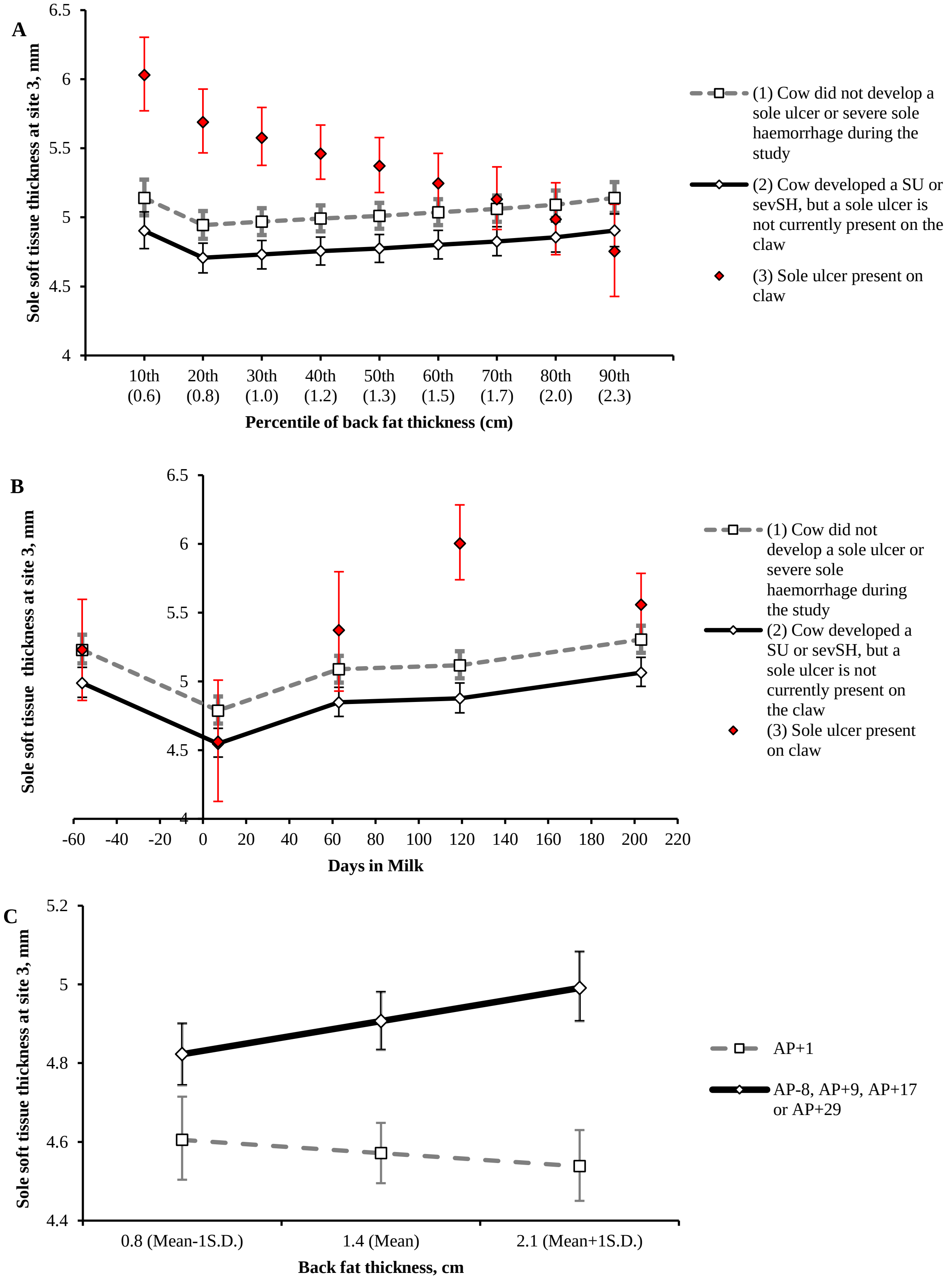

Skin
Subcutaneous fat
Fascia superficialis
Interfascial fat
Fascia profunda
Gluteus medius
Distal phalanx
DC
Distal phalanx
Digital cushion
Corium
Horn

Figure 2:



Sole soft tissue thickness, mm
Back fat thickness, mm
Body condition score
10
9
8
7
6
5
4
3
2
1
0
30
25
20
15
10
5
0
1
1.5
2
2.5
3
3.5
4
4.5
5
Sole soft tissue thickness at Site 1 (corium only)
Sole soft tissue thickness at Site 2
Sole soft tissue thickness at Site 3
Back fat thickness, cm

Figure 3:



A
Sole soft tissue thickness at site 3, mm
6.5
6
5.5
5
4.5
4
10th (0.6)
20th (0.8)
30th (1.0)
40th (1.2)
50th (1.3)
60th (1.5)
70th (1.7)
80th (2.0)
90th (2.3)
Percentile of back fat thickness (cm)
(1) Cow did not develop a sole ulcer or severe sole haemorrhage during the study
(2) Cow developed a SU or sevSH, but a sole ulcer is not currently present on the claw
(3) Sole ulcer present on claw
B
Sole soft tissue thickness at site 3, mm
6.5
6
5.5
5
4.5
4
-60
-40
-20
0
20
40
60
80
100
120
140
160
180
200
220
Days in Milk
(1) Cow did not develop a sole ulcer or severe sole haemorrhage during the study
(2) Cow developed a SU or sevSH, but a sole ulcer is not currently present on the claw
(3) Sole ulcer present on claw
C
Sole soft tissue thickness at site 3, mm
5.2
5
4.8
4.6
4.4
0.8 (Mean-1S.D.)
1.4 (Mean)
2.1 (Mean+1S.D.)
Back fat thickness, cm
AP+1
AP-8, AP+9, AP+17 or AP+29